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INVESTIGATION OF IMMUNOREGULATORY ALPHAGLOBULIN (IRA).(U)
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Investigation of Immunoregulatory Alphaglobulin (IRA)

FINAL REPORT
(for the period 1967 – 1974)

Oct [redacted] 1975

97 p.

By

John A. Mannick, M. D.

Supported by

U. S. Army Medical Research and Development Command
Washington, D.C. 20314

Contract No. DA-49-193-MD-2621

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Boston, Massachusetts 02118

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061 325

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* Investigation of Immunoregulatory Alphaglobulin (IRA)		5. TYPE OF REPORT & PERIOD COVERED Final Report 1967-1974 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John A. Mannick, M. D.		8. CONTRACT OR GRANT NUMBER(s) DA 49-193-MD 2621
9. PERFORMING ORGANIZATION NAME AND ADDRESS Boston University Medical Center University Hospital Boston, Massachusetts 02118		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62110A 3A162110A821.00.041
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Cmd. Washington, D . C. 20314		12. REPORT DATE October 1975 13. NUMBER OF PAGES 10 pages
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT *(of this Report)* A pproved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*		
20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*		

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

49-193-MD-2621

SUMMARY ANNUAL PROGRESS REPORTS

YEARS 1967-1974

Beginning in 1967 with the support of this contract, we were able to isolate by DEAE cellulose chromatography a suppressive alphaglobulin fraction from normal human plasma. We called this fraction immunoregulatory alphaglobulin (IRA) because it suppressed a wide variety of cellular immune responses in a number of animal species. This first work was essentially a confirmation of the earlier reports of Mowbray who had obtained similar material from bovine serum. The human IRA we had obtained suppressed the rejection of skin allografts in both rabbits and inbred mice (see enclosed bibliography). We also found that this material would suppress *in vitro* the stimulation of normal human peripheral blood lymphocytes by mitogens and specific antigens to which the lymphocyte donor had become sensitized. The suppressive alphaglobulin material was quite heterogeneous by electrophoresis, was not destroyed by heating at 56°C. and was apparently non toxic to a variety of mammalian cells *in vitro* as indicated by Trypan blue dye exclusion. It also had no evident toxic effects on experimental animals.

We next found that IRA-like activity was contained in Cohn fraction IV a byproduct of commerical blood fractionation laboratories. This Cohn fraction IV was then used as the starting material for future experiments designed to characterize the active fraction contained in the IRA. Over the succeeding three years we were able to show that IRA inhibited the plaque forming cell response mice to sheep red blood cells (SRBC) when

administered prior to the injection of antigen. It inhibited both the primary and secondary responses. IRA was found to be totally ineffective if injection followed the antigen. We discovered that IRA inhibition of peripheral blood lymphocytes in vitro could be entirely eliminated even after 96 hours exposure of the cells to IRA if they were washed thoroughly and then tested for stimulation. Thus, it appeared that IRA did not irreversibly affect lymphocyte function but appeared to act in a reversible fashion most likely on the cell membrane as indicated by its ability to prevent T cell rossette formation and recognition of antigen. We were also able to show that IRA had no effect upon B cell responses as indicated by its failure to inhibit the plaque forming cell response to E. coli lipopolysacchride 055:B5. IRA also had no effect on B cell (EAC) rossette formation by human peripheral blood lymphocytes. However, IRA did effect lymphokine production by lymphocytes as indicated by its inhibitory effect MIF production by sensitized guinea pig peritoneal lymphocytes exposed to specific antigens. IRA had no effect on macrophage immobilization once MIF was present in the macrophage culture medium, however.

In 1970 during attempts to purify IRA protein we discovered while we could concentrate activity in smaller and smaller fractions by gel filtration and ion exchange chromatography the specific activity of the final material was often no greater than that of the starting material. We then looked at our procedures and discovered that fractions at each step had been purified by dialysis. Earlier experiments had shown that at neutral pH dialysis of IRA protein failed to remove any detectable activity, However, we had

conducted a number of chromatographic experiments at pH's in the acid range and discovered that at acid pH low molecular weight material containing IRA activity was lost during dialysis. Investigation of this finding in detail yielded evidence that the majority of activity detectable in IRA protein could be recovered as a polypeptide fraction of less than 10,000 daltons following acidification of the protein and ultrafiltration on appropriate sized membranes or dialysis. We then further characterized the active peptide fraction and found that it contained no detectable sugar or nucleic acid. It was not affected by lipid solvents and contained no cortisol or prostaglandins. Chemically the material appeared to be entirely polypeptide as determined by the quantitative Biuret test. We found that IRA-peptide would perform biologically in a fashion entirely similar to IRA protein. This included inhibition of skin allograft rejection in mice, inhibition of immunity to experimental tumors in mice, inhibition of kidney allograft rejection in rats and inhibition T lymphocyte activation in vitro in mice and in man.

Over the years 1971-1974 we made a number of unsuccessful attempts to obtain pure IRA-peptide by repeated gel filtration and ion exchange column chromatography. The active fractions obtained at the end of such procedures were always heterogeneous by high voltage electrophoresis. The same failure obtained when we attempted to purify this material by polyacrylamide electrophoresis. In the analytical ultracentrifuge the active IRA-peptide preparations

obtained by gel filtration moved as a single peak with sedimentation coefficient of approximately 0.3S.

We have also attempted several times to make antibody to IRA-peptide in the rabbit by coupling this material to rabbit serum albumin by the carbodiimide method. We have obtained antibody which will react with crude IRA-peptide coupled to rabbit serum albumin in gel defusion assays, however, this antibody has apparently minimal reactivity to the active IRA preparation and will remove little of the IRA activity from impure preparations.

In the 1973-1974 grant year we began a study of approximately 40 patients who were subjected to various degrees of trauma including surgery and burns. We have found that approximately 60% of the entire group of trauma patients have immunosuppressive serum, defined as serum which will inhibit by 50% or more the <u>in vitro</u> stimulation of normal human peripheral blood lymphocytes by optimum doses of phytohemaglutinin (PHA) when compared with normal human serum or autologous serum obtained from the lymphocyte donors. The immunosuppressive activity in trauma patient serum does not appear to be caused by anesthestic agents since patients undergoing general anesthesia for minor surgery do not develop suppressive serum and many traumatized or septic patients have suppressive serum without receiving any anesthesia. The suppressive activity also did not correlate with barbituate or with cortisol levels in the serum of these individuals. We fractionated the serum of several of these individuals by DEAE cellulose chromatography and found that the majority of the immunosuppressive activity was recovered in the first protein peak, whereas immunosuppressive activity in the serum

of normal patients is recovered in later alphaglobulin-rich peaks. We have also found that the immunosuppressive activity contained in Peak I protein from trauma serum can be recovered as a peptide fraction of less than 10,000 molecular weight after diafiltration. This peptide is apparently either unbound or loosely bound to the protein in Peak I. The peptide obtained by diafiltration is very highly suppressive of T lymphocyte function both in vivo and in vitro. It appears, therefore, that patients who have been recently traumatized have high levels of a circulating immunosuppressive peptide fraction which appears to be similar to IRA-peptide. This trauma peptide has also been investigated chemically and has been found to be entirely composed of polypeptide as far as can be determined by the quantitative Biuret test. The presence of nucleic acid and carbohydrate has been ruled out by standard chemical techniques and no cortisol is present as determined by the competitive protein binding assay. Similarly, the trauma peptide contains no prostaglandins E1 and E2 as determined by radioimmunoassay.

It thus appears likely that following major trauma or burns a substantial number of patients develop immunosuppressive serum which inhibits T lymphocyte function. The immunosuppressive activity in such serum is apparently largely contained in a peptide fraction resembling IRA-peptide found in trace amounts in normal serum. Trauma serum, however, contains from five to ten times, by weight, the amount of IRA-like activity in normal serum.

John A. Marzouk, M.D.
Principal Investigator 10/1/75

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